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G-proteins of fat-cells

Role in hormonal regulation of intracellular inositol 1,4,5-trisphosphate

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Pertussis toxin abolishes hormonal inhibition of adenylate cyclase, hormonal stimulation of inositol 1,4,5-trisphosphate accumulation in rat fat-cells, and catalyses the ADP-ribosylation of two peptides, of M_r 39000 and 41000 [Malbon, Rapiejko & Mangano (1985) J. Biol. Chem. **260**, 2558–2564]. The 41000-M_r peptide is the α-subunit of the G-protein, referred to as G_i, that is believed to mediate inhibitory control of adenylate cyclase by hormones. The nature of the 39000-M_r substrate for pertussis toxin was investigated. The fat-cell 39000-M_r peptide was compared structurally and immunologically with the α-subunits of two other G-proteins, G_t isolated from the rod outer segments of bovine retina and G_o isolated from bovine brain. After radiolabelling in the presence of pertussis toxin and [^{32}P]NAD$^+$, the electrophoretic mobilities of the fat-cell 39000-M_r peptide and the α-subunits of G_o and G_t were nearly identical. Partial proteolysis of these ADP-ribosylated proteins generates peptide patterns that suggest the existence of a high degree of homology between the fat-cell 39000-M_r peptide and the α-subunit of G_o. Antisera raised against purified G-proteins and their subunits were used to probe immunoblots of purified G_t, G_i, G_o, and fat-cell membrane proteins. Although recognizing the 36000-M_r β-subunit band of G_t, G_i, G_o and a 36000-M_r fat-cell peptide, antisera raised against G_t failed to recognize either the 39000- or the 41000-M_r peptides of fat-cells or the α-subunits of G_o and G_i. Antisera raised against the α-subunit of G_o, in contrast, recognized the 39000-M_r peptide of rat fat-cells, but not the α-subunit of either G_i or G_t. These data establish the identity of G_o, in addition to G_i, in fat-cell membranes and suggest the possibility that either G_o or G_i alone, or both, may mediate hormonal regulation of adenylate cyclase and phospholipase C.

INTRODUCTION

Heterotrimeric GTP-binding regulatory proteins (G-proteins) transduce information from signal-recognition cell-surface membrane proteins to specific effector molecules. Hormones bind to specific receptors and regulate the stimulation and inhibition of adenylate cyclase via the G_s and G_i proteins, respectively (for references, see Gilman, 1984). Light activates the photopigment rhodopsin in bovine retinal rods and modulates cyclic GMP phosphodiesterase activity via G_t (referred to as 'transducin'; Stryer *et al.*, 1981). In a similar fashion G-proteins appear to mediate phosphatidylinositol breakdown in response to activation of specific hormone receptors in some tissues (for references, see Joseph, 1985) and in phototransduction in invertebrates (Fein *et al.*, 1984; Brown *et al.*, 1984). The β- and γ-subunits of several of these G-proteins are highly homologous (Manning *et al.*, 1984; Cerione *et al.*, 1985). The α-subunits bind GTP, express GTPase activity, and are specifically ADP-ribosylated in the presence of NAD$^+$ and bacterial enterotoxins (Gilman, 1984). Cholera toxin catalyses the ADP-ribosylation of the α-subunit of G_s (Gill & Meren, 1978), the G-protein that transduces stimulation of adenylate cyclase (Northup *et al.*, 1980), whereas pertussis toxin acts on the α-subunit of G_i (Katada & Ui, 1982), the G-protein that transduces inhibition of the adenylate cyclase (Bokoch *et al.*, 1984; Codina *et al.*, 1983). Both toxins catalyse ADP-ribosylation of the α-subunit of the rod-outer-segment G-protein, G_t (Abood *et al.*, 1982; Van Dop *et al.*, 1984).

In contrast with an earlier report identifying only a 41000-M_r substrate for pertussis toxin in the rat fat-cell (Murayama & Ui, 1983), we observed the presence of a 39000-M_r peptide in fat-cell membranes that, like the α-subunit of G_i, was specifically ADP-ribosylated in the presence of pertussis toxin and [^{32}P]NAD$^+$ (Malbon *et al.*, 1984). This peptide was homologous to, but not identical with, the α-subunit of G_i and its labelling by pertussis toxin was influenced by guanine nucleotides. These data suggested that this 39000-M_r peptide may be the α-subunit of another G-protein, different from G_i. A 39000-M_r pertussis-toxin substrate has been identified in high abundance in bovine brain and shown to be the α-subunit of a G-protein, referred to as G_o (Sternweis & Robishaw, 1984; Neer *et al.*, 1984). Although designating the M_r of the two substrates for pertussis-toxin-catalysed

Abbreviations used: G-protein, guanine nucleotide-binding regulatory protein; G_s, G-protein mediating stimulatory control of adenylate cyclase; G_i, G-protein mediating inhibitory control of adenylate cyclase; G_o, the G-protein of bovine brain with a 39000-M_r α-subunit that can be ADP-ribosylated by pertussis toxin; G_t, the G-protein of rod outer segments of bovine retina with a 39000-M_r α-subunit that can be ADP-ribosylated by pertussis toxin and cholera toxin.

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ADP-ribosylation as 41000 and 40000 in previous reports, we assign the fat-cell peptides M_r values of 41000 and 39000 in the present work, on the basis of their migration relative to that of α-subunits of G_i, G_o and G_t. In the present paper, the 39000-M_r pertussis-toxin substrate of fat-cells is compared structurally and immunologically with the α-subunits of G_o, G_i and G_t. The data demonstrate the existence of G_o in a non-neural tissue and raise the possibility that regulation of adenylate cyclase or phosphatidylinositol breakdown by hormones is mediated by G_i, G_o, or perhaps both.

EXPERIMENTAL

Preparation of fat-cell plasma membranes

Highly purified rat fat-cell plasma membranes were prepared from parametrial adipose tissue isolated from female Sprague–Dawley rats (SD strain) weighing 150–175 g by the method of McKeel & Jarett (1970) and stored at −80 °C until use. Plasma membranes were prepared in buffers containing the following proteinase inhibitors: aprotinin (0.5 μg/ml), leupeptin (0.5 μg/ml), and 100 μM-phenylmethanesulphonyl fluoride.

Purification of G-proteins

The rod-outer-segment G-protein, G_t, was purified from bovine retinae by the method of Kuhn (1980), except that all procedures were performed under normal laboratory illumination. Bovine brain G_i and G_o were prepared as a mixture of both proteins, as described by Sternweis & Robishaw (1984). This procedure yields fractions of pure G_o α-subunit as well as those containing α-subunits of G_o and G_i with the β–γ-subunit complex. This mixture is designated as 'G_i, G_o' in the present work.

Pertussis-toxin-catalysed ADP-ribosylation

Fat-cell membranes (50–300 μg of protein) were incubated in the presence of 1.0 μg of pertussis toxin and [^{32}P]NAD$^+$ in a final volume of 0.1 ml and subjected to SDS/polyacrylamide-gel electrophoresis as described previously (Malbon *et al.*, 1985*b*). Purified G_t and 'G_i, G_o' were incubated in the presence of 0.1 μg of pertussis toxin in a final volume of 10 μl. The reaction was terminated by the addition of 40 μl of sample buffer containing 20% (v/v) 2-mercaptoethanol, and the mixture was subjected to electrophoresis as described above.

Preparation of antisera

Polyclonal antisera were raised against oligomeric G_t or α-subunit of G_o by multi-site intradermal injection in rabbits (Vaitukaitis *et al.*, 1971); a total of 50 μg of α-subunit of G_t or G_o in complete Freund's adjuvant was injected over 20–30 sites, followed at 3 weeks by injection in incomplete Freund's adjuvant at sites of inflammation. Antibody titres were monitored by nitrocellulose blots made visible as described below.

Immunoblotting of SDS/polyacrylamide gels

Peptides were subjected to gel electrophoresis and then transferred from the gel to nitrocellulose in a Bio-Rad Trans-Blot apparatus, by the procedure of Towbin *et al.* (1979), as modified by Erickson *et al.* (1982). Immunoblots were probed with antisera at dilutions of 1:200 in phosphate-buffered saline (20 mM-sodium phosphate/150 mM-NaCl, pH 7.2) containing 0.3% Tween-20 and made visible using a calf alkaline phosphatase-conjugated second antibody as described by Smith & Fisher (1984).

Partial proteolytic digestion of ADP-ribosylated G-proteins

Purified bovine brain 'G_i, G_o' as well as rat fat-cell membranes that were radiolabelled in the presence of [^{32}P]NAD$^+$ and pertussis toxin were digested by partial proteolysis by the method of Cleveland *et al.* (1977), as modified by Hudson & Johnson (1980). The proteolysed peptides were separated on 14–18%-polyacrylamide gradient gels.

Measurement of inositol 1,4,5-trisphosphate in fat-cells

Isolated fat-cells were incubated in Krebs–Ringer phosphate buffer (Malbon *et al.*, 1978) containing 4% (w/v) bovine serum albumin and [^{3}H]inositol (0.2–0.5 mCi/ml) in a shaking water bath at 37 °C for 3 h. The cells were washed extensively with Krebs–Ringer phosphate buffer and then incubated with and without hormones for 20 min. At the end of the incubation the cells were washed and treated with ice-cold chloroform/methanol/HCl (80:40:1, by vol.). Inositol phosphates were analysed by ion-exchange h.p.l.c. as described by Irvine *et al.* (1985).

Protein determination

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Materials

Except for *myo*-[2-^{3}H]inositol, purchased from Amersham, all radiochemicals were purchased from New England Nuclear. Pre-stained M_r standards were purchased from Bethesda Research Laboratories. All other

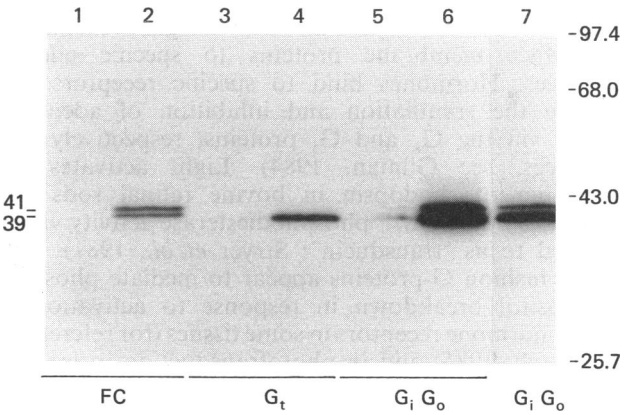

Fig. 1. Polyacrylamide-gel analysis of rat fat-cell membranes, G_t and bovine brain 'G_i, G_o' after ADP-ribosylation in the presence of pertussis toxin and [^{32}P]NAD$^+$

Rat fat-cell membranes (FC; 50 μg; lanes 1 and 2), G_t (250 ng; lanes 3 and 4) and bovine brain 'G_i, G_o' (250 ng, lanes 5, 6 and 7) were incubated in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4, 6 and 7) of pertussis toxin and [^{32}P]NAD$^+$ and subjected to SDS/polyacrylamide-gel electrophoresis as described in the Experimental section. The gel was exposed to Kodak XAR-5 film for 6 h. Lane 7 represents a 12 h exposure of lane 6 of this same gel. Values to the right and left represent M_r ($\times 10^{-3}$).

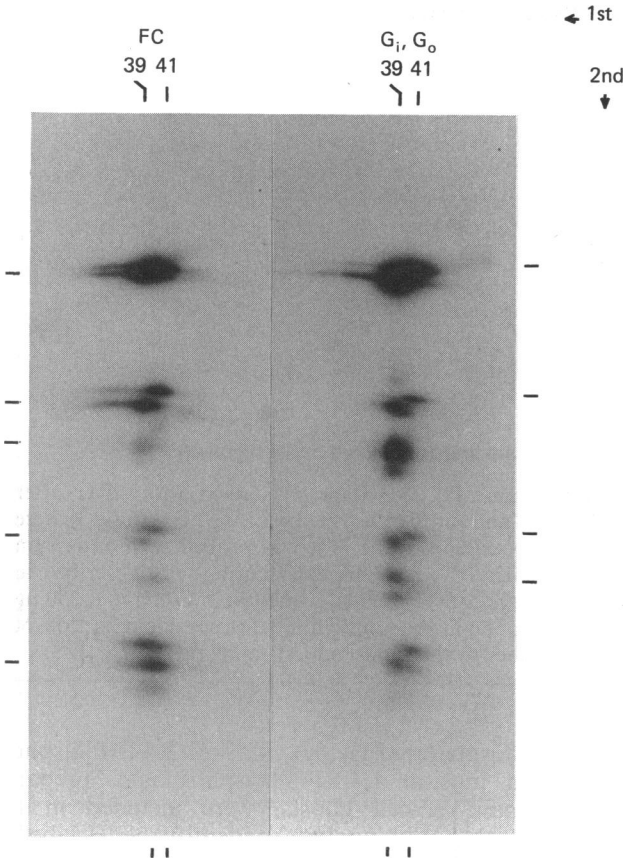

Fig. 2. Partial proteolytic digestion of pertussis-toxin targets from rat fat-cell membranes and purified bovine brain 'G_i, G_o'

Rat fat-cell membranes (FC; 275 μg; left-hand panel) and purified bovine brain 'G_i, G_o' (130 ng; right-hand panel) were incubated in the presence of pertussis toxin and [^{32}P]NAD$^+$ and subjected to SDS/polyacrylamide (10%)-gel electrophoresis on a 1.5 mm-thick slab gel (first dimension, '1st'). The regions of the gel corresponding to peptides with M_r 45000–20000 (identified by pre-stained M_r standards) were excised and bound to the stacking gel of a 14–18%-polyacrylamide gradient gel with a 1% agarose solution containing 8.0 μg of elastase. Proteolysis and subsequent electrophoresis (second dimension, '2nd') were performed as described in the Experimental section. The autoradiogram shown represents a 4-day exposure for the fat-cell lane and a 18 h exposure for the lane containing 'G_i, G_o'.

reagents were purchased from standard commercial suppliers.

RESULTS

The electrophoretic mobilities of the 39000- and 41000-M_r pertussis-toxin substrates of rat fat-cell membranes were compared with those of the α-subunits of G_i, G_o and G_t. Fat-cell membranes or purified G-proteins were incubated with pertussis toxin and [^{32}P]NAD$^+$ and then subjected to electrophoresis on polyacrylamide gels in the presence of SDS and a disulphide-bridge-reducing agent. An autoradiogram of the ADP-ribosylated peptides is shown in Fig. 1. The 41000-M_r fat-cell peptide displays a mobility similar to that of G_i α-subunit. The 39000-M_r fat-cell peptide displays a mobility similar to that of G_o and G_t α-subunits. In the experiment illustrated in Fig. 1, G_t α-subunit appears to migrate more rapidly than the fat-cell 39000-M_r peptide and G_o α-subunit. This is not a consistent observation; identical migration of α-subunits of G_t and G_o has been observed. The apparent difference in separation between fat-cell G_i-subunit and 39000-M_r peptide as compared with bovine G_i and G_o α-subunits are most probably due to differences in the protein loading required to give comparable abundance of fat-cell G_i and 39000-M_r peptide (50 μg and 250 ng respectively).

The extent of homology that exists between the 39000-M_r fat-cell peptide and G_o α-subunit was investigated by comparing the electrophoretic patterns of proteolytic fragments derived from these peptides. Previously we demonstrated that the 39000-M_r and 41000-M_r substrates for pertussis toxin in fat-cell membranes were homologous, but non-identical, peptides by this same approach (Malbon *et al.*, 1984). Fat-cell membranes as well as bovine brain 'G_i, G_o' were ADP-ribosylated in the presence of [^{32}P]NAD$^+$ and pertussis toxin, separated in the first dimension on polyacrylamide gels and subjected to partial digestion with a proteinase. The resultant proteolytic fragments were separated by polyacrylamide-gel electrophoresis in the second dimension. Fig. 2 is an autoradiogram of partial peptide maps generated by the action of elastase.

Striking similarities exist between the peptide maps of fat-cell pertussis-toxin substrates and of α-subunits of bovine brain 'G_i, G_o'. The peptide fragments generated by elastase from the 39000-M_r fat-cell toxin substrate were compared with those generated from G_o α-subunit; undigested G_o α-subunit and four fragments of it were observed in the digests of fat-cell 39000-M_r peptide (denoted by marks in the left-hand margin of Fig. 2). Digests of fat-cell 39000-M_r toxin substrate, as compared with those of G_o α-subunit by α-chymotrypsin, displayed undigested peptides and three fragments in common; digests by *Staphylococcus aureus* V-8 protease displayed three proteolytic fragments common to both G_o α-subunit and fat-cell 39000-M_r toxin substrate (results not shown). As previously observed (Malbon *et al.*, 1984), the peptide maps of 39000-M_r and 41000-M_r fat-cell peptides were homologous but non-identical, as were those of α-subunits of G_o and G_i of bovine brain (Fig 2). Peptides of partial digests of fat-cell 41000-M_r toxin substrate by elastase displayed the same mobilities as the undigested G_i α-subunit and three fragments derived from this peptide (denoted by marks on the right-hand margin of Fig. 2). Digests of fat-cell 41000-M_r toxin substrate, as compared with G_i α-subunit, by α-chymotrypsin displayed undigested peptides and three additional fragments in common; digests by *S. aureus* V-8 protease displayed six peptide fragments derived from fat-cell 41000-M_r peptide and G_i α-subunit in common (results not shown). These data suggest that a high degree of homology exists between the fat-cell 39000-M_r substrate for pertussis toxin and G_o α-subunit, as well as between the fat-cell 41000-M_r peptide and G_i α-subunit.

The extent to which the G-proteins of rat fat-cells are homologous to G_o, G_i, and G_t was investigated by an immunological approach. Fat-cell membranes and purified G_o, G_i and G_t were subjected to electrophoresis

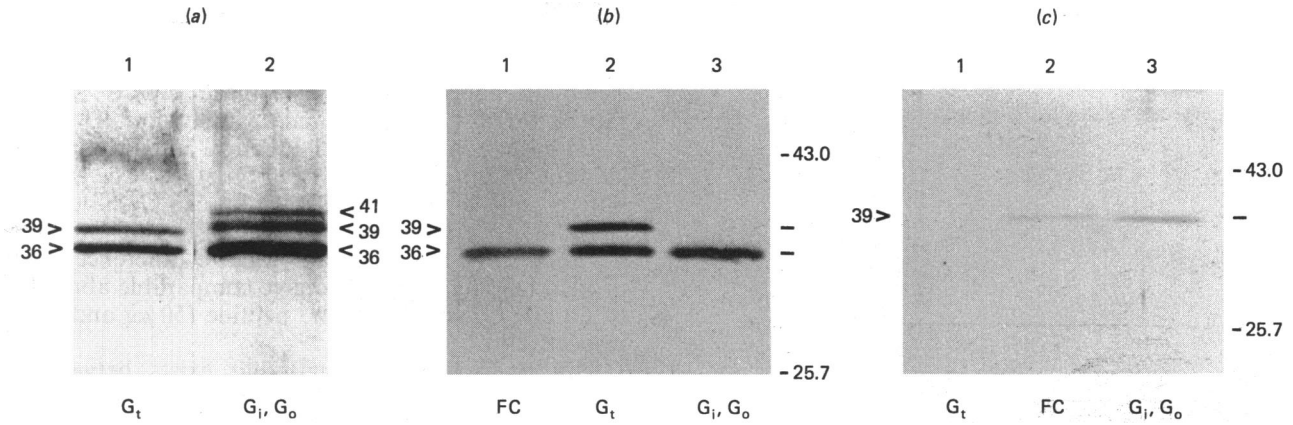

Fig. 3. Immunoblot analysis of G-proteins from rat fat-cell membranes, bovine brain and retinal rod outer segment

(*a*) Polyacrylamide gel of 0.5 μg of G_t (lane 1) and 0.5 μg of bovine brain 'G_i, G_o' (lane 2) stained with silver after electrophoresis. (*b*) Rat fat-cell membranes (lane 1; FC), purified G_t (lane 2), and purified bovine brain 'G_i, G_o' (lane 3) were subjected to SDS/polyacrylamide-gel electrophoresis and transferred to nitrocellulose. The blot was probed with antiserum raised against G_t (*b*) and then stained with a calf alkaline phosphatase-conjugated goat anti-rabbit antibody as described in the Experimental section. (*c*) A parallel blot was probed with antiserum raised against the purified α-subunit of G_o (lane 1, 250 ng of G_t; lane 2, 275 μg of rat fat-cell membranes; lane 3, 250 ng of 'G_i, G_o'). The approximate limits for specific detection of β- and α-subunits of G_o are 3 and 30 ng respectively with these antisera. Values to the right and left represent M_r ($\times 10^{-3}$).

on polyacrylamide gels in the presence of SDS under reducing conditions, and then electrophoretically transferred to nitrocellulose. The nitrocellulose blots were then probed with antisera raised against purified G-proteins or specific subunits isolated from these proteins. Fig. 3(*b*) shows blots of fat-cell membranes (lane 1), G_t (lane 2) and 'G_i, G_o' (lane 3) probed with an antiserum raised against G_t. This antiserum recognized both the α (39000-M_r) and β (36000-M_r) subunits of G_t, the upper band from β-subunit of 'G_i, G_o', and a 36000-M_r peptide of fat-cell membranes. However, the antiserum did not cross-react with the α-subunits of an equivalent amount (0.25 μg of protein) of 'G_i, G_o' or the 39000- and 41000-M_r peptides of fat-cell membranes. The lack of cross-reactivity between the antibodies raised against G_t α-subunit and the α-subunits of 'G_i, G_o' appears to reflect the specificity of the antibodies and not the relative amount of G-proteins employed in the blotting.

Antiserum raised against purified G_o α-subunit of bovine brain was employed to probe blots of fat-cell membrane proteins, purified 'G_o, G_i' and G_t (Fig. 3*c*). The antiserum raised against G_o α-subunit of bovine brain recognized a 39000-M_r peptide of rat fat-cells (lane 2), G_o α-subunit (lane 3), but not G_t α-subunit (lane 1). A second antiserum raised against G_o α-subunit of bovine brain also recognized the 39000-M_r peptide of rat fat-cells, but not the α-subunits of G_i or G_t (results not shown).

The influence of pertussis-toxin treatment on catecholamine-stimulated generation of inositol phosphates by rat fat-cells was investigated, since it has been reported that it is the action of this toxin on G_i specifically that results in its effects on hormonal regulation of phosphatidylinositol metabolism (Mills & Fain, 1985). Fat-cells were isolated from control and pertussis-toxin-treated rats, metabolically labelled with [^{3}H]inositol, and then challenged with adrenaline. H.p.l.c. separation of the [^{3}H]inositol phosphates is shown in Fig. 4. Adrenaline stimulates the generation of several inositol phosphates (Fig. 4*a*). Inositol 1,4-[^{32}P]bisphosphate and inositol 1,4,5-[^{32}P]trisphosphate, prepared from human red-cell ghosts, were included in the samples before h.p.l.c. analysis to identify the ^{3}H-labelled inositol phosphates (results not shown). Inositol monophosphate (results not shown), bisphosphate and trisphosphate were generated in response to adrenaline. In the presence of the β-adrenergic antagonist (−)-propranolol (10 μM), inositol phosphate, bisphosphate and trisphosphate were still generated in rat fat-cells stimulated by 10 μM-adrenaline (results not shown). Fat-cells isolated from pertussis-toxin-treated rats displayed markedly diminished generation of inositol trisphosphate in response to stimulation with adrenaline (Fig. 4*b*). Pertussis-toxin treatment did result in the generation of two unidentified peaks of ^{3}H labelling in response to catecholamine, rather than inositol trisphosphate. These are not inositol mono- and bisphosphate, and their identities have not yet been determined.

DISCUSSION

Pertussis toxin, like cholera toxin, has proved to be an invaluable probe for the study of the structure and biology of G-proteins. Treatment with pertussis toxin modifies many cellular responses that are believed to be mediated by G-proteins, including hormonal regulation of cyclic AMP (reviewed by Gilman, 1984) and of phosphatidylinositol metabolism (reviewed by Joseph, 1985). In virtually all tissues and cells that have been used as sources of membranes, pertussis toxin has been reported to catalyse the ADP-ribosylation of a single predominant 41000-M_r peptide (for references see Malbon *et al.*, 1984). On the basis of the ability of pertussis toxin to attenuate the inhibitory control of cyclic AMP concentrations by hormones in many of these systems and the presence of only a single 41000-M_r substrate for toxin-catalysed ADP-ribosylation, the heterotrimeric G-protein that possesses this 41000-M_r

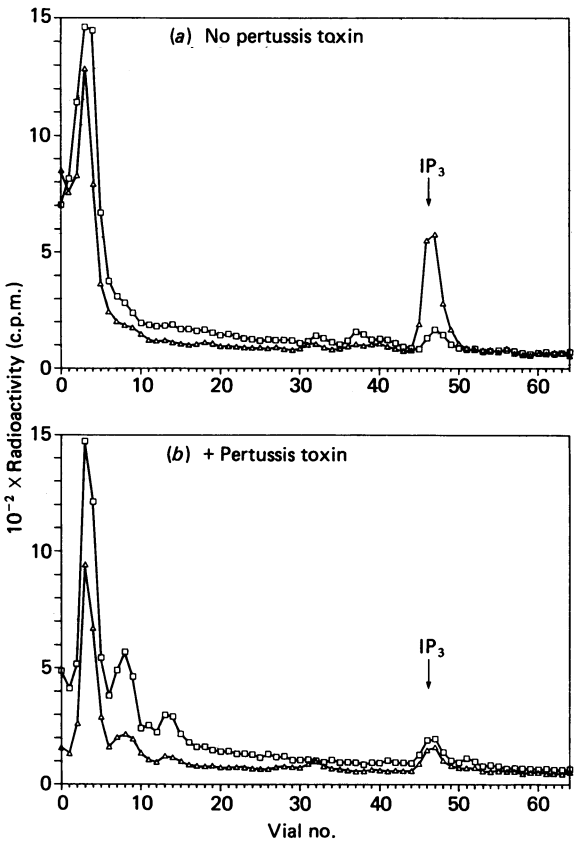

Fig. 4. Inositol 1,4,5-trisphosphate accumulation in fat-cells from control and pertussis-toxin-treated rats: response to adrenaline

Rats were injected intraperitoneally with 50 μg of pertussis toxin or vehicle. At 30 h after the injection, fat-cells were isolated from the rats and then metabolically labelled with [^{3}H]inositol as described in the Experimental section. The cells were washed and then incubated with (△) or without (□) 10 μM-adrenaline for 20 min at 37 °C. Inositol phosphates were analysed as described in the Experimental section. Inositol incorporation into total lipids was the same in fat-cells from control and toxin-treated rats. The peak of inositol 1,4,5-trisphosphate (IP_3) was identified by co-chromatography of inositol 1,4,5-[^{32}P]trisphosphate prepared from human red cells. Peaks appearing in vials 3–4 represent inositol 1,4-bisphosphate; the fractions containing inositol monophosphate (results not shown) occurred at earlier times in the separation.

α-subunit was termed G_i. It has been suggested that effector systems other than adenylate cyclase that display sensitivity to the actions of pertussis toxin are also regulated via G_i (Kurose & Ui, 1985; Mills & Fain, 1985).

There are several prominent exceptions to the general observation of a single 41000-M_r substrate for pertussis-toxin action in cell membranes. First, the α-subunit of G_t that is ADP-ribosylated in the presence of pertussis toxin and [^{32}P]NAD^+ is a 39000-M_r peptide (Stryer *et al.*, 1981; Van Dop *et al.*, 1984). The α-subunits of G_t and G_i are highly homologous, but non-identical, peptides (Manning & Gilman, 1983). Second, Sternweis & Robishaw (1984) and Neer *et al.* (1984) reported the isolation of a G-protein found in high abundance in bovine brain that, too, displays a 39000-M_r α-subunit. The 39000-M_r α-subunit of this 'other' G-protein, termed G_o, is specifically ADP-ribosylated in the presence of pertussis toxin and [^{32}P]NAD^+. Additionally rat adipocytes and chick myocytes display not one, but two, peptides of M_r 41000 and 39000 that are specifically ADP-ribosylated by the action of pertussis toxin (Malbon *et al.*, 1984; Halvorsen & Nathanson, 1984).

These observations were striking in several ways. We note that our findings differ from those of Murayama & Ui (1983), who reported the presence of only the 41000-M_r substrate for pertussis-toxin-catalysed labelling of rat fat-cells. Additionally, Kurose & Ui (1983) found only a 41000-M_r substrate for pertussis toxin in isolated myocytes. On the basis of their finding of only this single 41000-M_r toxin target, they concluded that the sole site of pertussis-toxin action is the G-protein, G_i, that is required for transmission of inhibitory signals from receptors to adenylate cyclase (Murayama & Kurose, 1983). Except for the retina and the bovine brain, a 39000-M_r substrate for toxin action had not been generally observed in other work on mammalian cells and tissues.

We report herein several observations critical to the understanding of the biology of G-proteins. First, we conclude that the rat fat-cell membrane contains both G_o and G_i, on the basis of both comparison of partial proteolytic peptide maps of toxin-catalysed radiolabelled α-subunits and the use of specific antisera raised against purified G-proteins and their subunits. It is no longer possible to use the effects of pertussis toxin on the cell biology to assign the effector system(s) that these G-proteins may regulate uniquely to G_i or G_o, because both of these G-proteins are ADP-ribosylated by the action of pertussis toxin.

Second, we have now shown that pertussis-toxin treatment nearly abolishes the ability of adrenaline to stimulate accumulation of inositol 1,4,5-trisphosphate in rat fat-cells, in addition to its ability to attenuate or abolish inhibitory control of adenylate cyclase by hormones (Murayama & Ui, 1983; Olansky *et al.*, 1983; Malbon *et al.*, 1985*b*). These data are in agreement with the report of Moreno *et al.* (1983) that the stimulatory effects of adrenaline on ^{32}P uptake into phosphatidylinositol of isolated rat fat-cells (an index of phosphatidylinositol metabolism) was abolished by pertussis-toxin treatment. Unique assignment of G_i or G_o as the mediator(s) of one or the other systems is no longer possible because effector systems controlling both cyclic AMP and phosphatidylinositol metabolism are modified by pertussis-toxin treatment. In adipocytes either G_i or G_o, alone or together, may mediate the effects of hormones on either the inhibitory control of adenylate cyclase or the control of phospholipase C, or both.

Several laboratories have reported on the use of pertussis toxin to dissect the transduction of cellular responses to 'calcium-mobilizing' hormones. The data derived from studies of neutrophils (Bokoch & Gilman, 1984; Okajima & Ui, 1984), HL-60 (Brandt *et al.*, 1985) and mast cells (Nakamura & Ui, 1985) suggest that hormonal regulation of phospholipase C is mediated by a pertussis-toxin substrate(s). However, data for the pituitary-derived GH_3 cells (Martin *et al.*, 1986), rat hepatocytes (Uhing *et al.*, 1985) and astrocytoma 1321N1 (Masters *et al.*, 1985) show no effect of

pertussis-toxin treatment on the phospholipase C regulation. Our data for rat adipocytes would suggest that they contain a transduction pathway(s) in common to neutrophils and mast cells which is modifiable by pertussis toxin. Neither neutrophils nor mast cells appear to contain G_o protein, as assessed by pertussis-toxin-catalysed labelling.

Finally, the inability to detect the presence of G_o via pertussis-toxin-catalysed ADP-ribosylation does not necessarily mean that G_o is absent. Owens *et al.* (1985) confirmed the existence of a 39000-M_r substrate for pertussis toxin in the rat fat-cell. Our previous studies of the ADP-ribosylation of the 39000- and 41000-M_r peptides of fat-cells by the action of pertussis toxin suggested that the toxin acts not on the free α-subunit, but rather on the α-subunit complexed with β- and γ-subunits (Malbon *et al.*, 1984). Huff & Neer (1986) reported that G_o α-subunit of bovine brain associates less readily than G_i α-subunit with β- and γ-subunits. Thus the failure to detect G_o α-subunit in membranes incubated with pertussis toxin and labelled NAD^+ may reflect an insufficient amount of β-/γ-subunit complex to support toxin-catalysed labelling of G_o α-subunit, rather than the absence of G_o. These observations suggest that G_o may exist in many tissues heretofore believed to possess only G_i. α-Subunits of both G_i and G_o have been identified in bovine (Mumby *et al.*, 1986) and rabbit (Malbon *et al.*, 1985*a*) heart preparations, providing additional evidence to support our conjecture.

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